

IN THE CLAIMS

Please amend the claims of this application as indicated in the listing below, which replaces all previous listing of claims.

1. (Currently Amended) An enzyme mixture comprising a first enzyme and a second enzyme, wherein said first enzyme ~~comprises 5'-3' polymerization activity of~~ is a DNA polymerase or reverse transcriptase, and said second enzyme is Pfu DNA polymerase, except that it is mutated consisting of an amino acid substitution at an amino acid position selected from the group consisting of: Y410, T542, D543, K593, Y595, Y385, G387, and G388, and wherein said second enzyme possesses 3'-5' exonuclease activity and reduced 5'-3' DNA polymerization activity.

2. (Cancelled)

3. (Previously Presented) The enzyme mixture of claim 2, wherein said first enzyme is selected from the group consisting of: Taq DNA polymerase, Tth DNA polymerase, U1Tma DNA polymerase, Tli DNA polymerase (Vent DNA polymerase), Pwo DNA polymerase, Tgo DNA polymerase, Pfu DNA polymerase, KOD DNA polymerase, JDF-3 DNA polymerase having the sequence shown in SEQ ID NO. 10, PGB-D DNA polymerase (Deep Vent DNA polymerase) and DP1/DP2 DNA polymerase.

4-9. (Cancelled)

10. (Currently Amended) The enzyme mixture of claim 1, wherein said Pfu DNA polymerase is mutated at one or more amino acid positions to create one or more amino acid substitutions, and the resulting ~~consists of~~ one or more amino acid substitutions are selected from the group consisting of: Y410F, T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P.

11. (Previously Presented) The enzyme mixture of claim 1, further comprising a PCR enhancing factor and/or an additive.

12. (Currently Amended) A kit comprising a first enzyme, a second enzyme, and packaging material therefor, wherein said first enzyme ~~comprises 5'-3' polymerization activity of~~ is a DNA polymerase or reverse transcriptase, said second enzyme is Pfu DNA polymerase, except that it is mutated ~~consisting of an amino acid substitution~~ at an amino acid position selected from the group consisting of: Y410, T542, D543, K593, Y595, Y385, G387, and G388, and wherein said second enzyme possesses 3'-5' exonuclease activity and reduced 5'-3' DNA polymerization activity.

13. (Cancelled)

14. (Previously Presented) The kit of claim 13, wherein said first enzyme is selected from the group consisting of: Taq DNA polymerase, Tth DNA polymerase, U1Tma DNA polymerase, Tli DNA polymerase (Vent DNA polymerase), Pwo DNA polymerase, Tgo DNA polymerase, Pfu DNA polymerase, KOD DNA polymerase, JDF-3 DNA polymerase having the sequence shown in SEQ ID NO. 10, PGB-D DNA polymerase (Deep Vent DNA polymerase) and DP1/DP2 DNA polymerase.

15-19. (Cancelled)

20. (Previously Presented) The kit of claim 12, further comprising one or more components selected from the group consisting of: a deoxynucleotide, a reaction buffer, a PCR enhancing factor and/or an additive, a control DNA template and a control primer.

21. (Cancelled)

22. (Currently Amended) The kit of claim 12, wherein said Pfu DNA polymerase is mutated at one or more amino acid positions to create one or more amino acid substitutions, and the resulting ~~consists of~~ one or more amino acid substitutions are selected from the group consisting of: Y410F, T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P.

23. (Withdrawn; Currently Amended) A method for DNA synthesis comprising:

(a) providing an enzyme mixture, said enzyme mixture comprising a first enzyme comprising a ~~DNA polymerization activity~~ that is a DNA polymerase or reverse transcriptase, and a second enzyme which is a mutant Pfu DNA polymerase comprising one or more mutations at amino acid positions selected from the group consisting of: D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388; and

(b) contacting said enzyme mixture with a nucleic acid template, wherein said enzyme mixture permits DNA synthesis.

24. (Withdrawn) The method of claim 23, wherein said nucleic acid template is a DNA molecule.

25. (Cancelled)

26. (Withdrawn; Currently Amended) The method of claim ~~[[25]]~~ 23, wherein said ~~DNA polymerase~~ first enzyme is selected from the group consisting of: Taq DNA polymerase, Tth DNA polymerase, U1Tma DNA polymerase, Tli DNA polymerase, Pfu DNA polymerase, KOD DNA polymerase, JDF-3 DNA polymerase, PGB-D DNA polymerase and DPL/DP2 DNA polymerase.

27-29. (Cancelled)

30. (Withdrawn) A method for DNA synthesis comprising:

- (a) providing an enzyme mixture, said enzyme mixture comprising a wild type Pfu DNA polymerase as a first enzyme, and a mutant Pfu DNA polymerase as a second enzyme which comprises a 3'-5' exonuclease activity and a reduced DNA polymerization activity, and
- (b) contacting said enzyme mixture with a nucleic acid template, wherein said enzyme mixture permits DNA synthesis.

31. (Withdrawn) A method for TA cloning of DNA synthesis product comprising:

- (a) providing an enzyme mixture, said enzyme mixture comprising a Taq DNA polymerase as a first enzyme, and a mutant Pfu DNA polymerase as a second enzyme which comprises a 3'-5' exonuclease activity and a reduced DNA polymerization activity;
- (b) contacting said enzyme mixture with a nucleic acid template, wherein said enzyme mixture permits DNA synthesis to generate a synthesized DNA product; and
- (c) inserting said synthesized DNA product into a TA cloning vector.

32. (Withdrawn) The method of claim 28, 30, or 31, wherein said mutant Pfu DNA polymerase comprises one or more mutations at amino acid positions selected from the group consisting of: D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388.

33. (Withdrawn) The method of claim 23, wherein said mutant Pfu DNA polymerase comprises one or more mutations selected from the group consisting of: D405E, Y410F, T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P.

34. (Withdrawn) The method of claim 23, 30 or 31, wherein said reaction mixture further comprises a PCR enhancing factor and/or an additive.

35. (Withdrawn) The method of claim 32, wherein said mutant Pfu DNA polymerase comprises one or more mutations selected from the group consisting of: D405E, Y410F, T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P.

36. (Currently Amended) An enzyme mixture comprising a first enzyme and a second enzyme, wherein said first enzyme is Taq DNA polymerase, and said second enzyme is Pfu DNA polymerase, except that it is mutated consisting of an amino acid substitution at an amino acid position selected ~~from amino acid positions~~ selected from the group consisting of: Y410, T542, D543, K593, Y595, Y385, G387, and G388, and wherein said second enzyme possesses 3'-5' exonuclease activity and reduced 5'-3' DNA polymerization activity.

37. (Currently Amended) The enzyme mixture of claim 36, wherein said Pfu DNA polymerase is mutated at one or more amino acid positions to create one or more amino acid

~~substitutions, and the resulting consisting of~~ one or more amino acid substitutions are selected from the group consisting of: Y410F, T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P.

38. (Currently Amended) The enzyme mixture of claim 36, wherein said Pfu DNA polymerase ~~consists of an amino acid substitution~~ is mutated at amino acid position G387.

39. (Currently Amended) The enzyme mixture of claim 36, wherein said Pfu DNA polymerase is mutated at amino acid position G387 and the resulting consists of the amino acid substitution is G387P.

40. (Currently Amended) An enzyme mixture comprising a first enzyme and a second enzyme, wherein said first enzyme is KOD DNA polymerase, and said second enzyme is Pfu DNA polymerase, except that it is mutated consisting of an amino acid substitution at an amino acid position selected from the group consisting of: D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388, and wherein said second enzyme possesses 3'-5' exonuclease activity and reduced 5'-3' DNA polymerization activity.

41. (Currently Amended) The enzyme mixture of claim 40, wherein said Pfu DNA polymerase comprises is mutated at one or more amino acid positions to create one or more

amino acid substitutions, and the resulting one or more amino acid substitutions are selected from the group consisting of: D405E, Y410F, T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P.

42. (Currently Amended) The enzyme mixture of claim 40, wherein said Pfu DNA polymerase ~~comprises an amino acid substitution~~ is mutated at amino acid position G387.

43. (Currently Amended) The enzyme mixture of claim 40, wherein said Pfu DNA polymerase ~~comprises the~~ is mutated at amino acid position G387 and the resulting amino acid substitution is G387P.

44. (Currently Amended) An enzyme mixture comprising a first enzyme and a second enzyme, wherein said first enzyme is a JDF-3 DNA polymerase having the sequence shown in SEQ ID NO. 10, and said second enzyme is Pfu DNA polymerase, except that it is mutated consisting of an amino acid substitution at an amino acid position selected from the group consisting of: D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388, and wherein said second enzyme possesses 3'-5' exonuclease activity and reduced 5'-3' DNA polymerization activity.



45. (Currently Amended) The enzyme mixture of claim 44, wherein said Pfu DNA polymerase is mutated at one or more amino acid positions to create one or more amino acid substituitions, and the resulting ~~consists of~~ one or more amino acid substitutions are selected from the group consisting of: D405E, Y410F, T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P.

46. (Currently Amended) The enzyme mixture of claim 44, wherein said Pfu-DNA polymerase ~~consists of an amino acid substitution~~ is mutated at amino acid position G387.

47. (Currently Amended) The enzyme mixture of claim 44, wherein said Pfu DNA polymerase ~~consists of the~~ is mutated at amino acid position G387 and the resulting amino acid substitution is G387P.

48. (Currently Amended) A kit comprising a first enzyme and a second enzyme, wherein said first enzyme is a Taq DNA polymerase, and said second enzyme is Pfu DNA polymerase, except that it is mutated consisting of an amino acid substitution in at least one an amino acid position selected from the group consisting of Y410, T542, D543, K593, Y595, Y385, G387, and G388, and packaging material therefor, and wherein said second enzyme possesses 3'-5' exonuclease activity and reduced 5'-3' DNA polymerization activity.

49. (Currently Amended) A kit comprising a first enzyme and a second enzyme, wherein said first enzyme is a KOD DNA polymerase, and said second enzyme is Pfu DNA polymerase, ~~except that it is mutated consisting of an amino acid substitution in at least one an~~ amino acid position selected from the group consisting of D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388, and packaging material therefor, and wherein said second enzyme possesses 3'-5' exonuclease activity and reduced 5'-3' DNA polymerization activity.

50. (Currently Amended) A kit comprising a first enzyme and a second enzyme, wherein said first enzyme is a JDF-3 DNA polymerase having the sequence shown in SEQ ID NO. 10, and said second enzyme is Pfu DNA polymerase, ~~except that it is mutated consisting of an amino acid substitution in at least one an~~ amino acid position selected from the group consisting of: D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388, and packaging material therefor, and wherein said second enzyme possesses 3'-5' exonuclease activity and reduced 5'-3' DNA polymerization activity.

51. (Previously Presented) The kit of claim 48, 49, or 50, wherein said kit further comprises a reagent selected from the group consisting of: dNTPs, reaction buffer, primer, and DNA enhancing factor.